Laboratory Investigations of the Reaction of Sulphate Ion in Anaerobic Media with Sulphate Reducing Bacteria in Sediments from the Field

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The bacterial reduction of sulphate ion in the calcareous sediments associated with the evaporative lake system of the Coorong, South Australia, results in the production of hydrogen sulphide. This is detected in the water from many of the piezometers in the region and is the pervading atmospheric odour.

Our early investigations of this reaction in the field and of field samples, revealed that detectable sulphide was present only in some piezometer waters. While the sulphate-sulphur was isotopically enriched above the $\delta^{34}S$ value for seawater (+21.0‰), to an extent which depended on the sample site, $\Delta^{34}S$ ($\delta^{34}S_{sulphate}$ - $\delta^{34}S_{sulphide}$) for water from piezometers where sulphide was detected, ranged from around 30 ‰ to greater than 60 ‰ (Batts *et al.,* 1996 & 1997a). Values of $\delta^{34}S$ and $\Delta^{34}S$ at any one site have varied little from one sampling to another over ten years. The highest $\Delta^{34}S$ values are consistently observed in piezometers where salt concentrations are high (up to ~ 5 times seawater values) and soluble sulphide species are at their highest concentrations. Rate studies have shown consistently that the highest $\Delta^{34}S$ values are observed where the rate of sulphate reduction to sulphide species is greatest (Bicknell *et al.,* 1997 & 1999).

Most Probable Number (MPN) counts for sulphate reducing bacteria (SRB) established that SRB are present at all sites in the study area although the MPN varied from site to site by a factor of 10^5. The growth of *Desulphovibrio* as the predominant sulphate reducing species was that most likely to be promoted by the selected media.

Observation of apparently anomalous ^{18}O enrichment in the sulphate ion from the field samples, subsequently explained (Batts *et al.*, 1997b), prompted the laboratory investigations of the reaction of sulphate ion, reported here. Initially, laboratory experiments were inoculated with SRB cultured from the field. The sulphur and oxygen isotope results from these experiments seemed not to offer any interpretation of the fieldwork data. As a consequence, it was decided that for laboratory work, sediments obtained from the field would be used in a microcosm to represent more closely the bacterial environment in which sulphate ion was reacting.

Fresh sediment samples from field sites were obtained from piezometer bottoms and kept refrigerated until used. The sites were selected to represent the spectrum of conditions related to previous observations of salinity and sulphate ion concentrations, soluble sulphide concentrations, bacterial counts and rate of conversion of sulphate to sulphide species. Two sets of SRB media suitable for the known characteristics of each site were prepared: the basic media and one in which the total salt concentration was adjusted to that specific for each site. On addition of the medium to each reaction vessel, the vessel was purged with nitrogen. To begin

each experiment, a known weight of sediment was added to the reaction vessel, usually 1 to 2 g L^{-1}, and the vessel again saturated with nitrogen. Some vessels were set aside and sampled only at the end of the reaction. Others were sampled for optical density and sulphate on 2 to 5 occasions during the experiment, using nitrogen positive pressure, and still others were purged continuously with a nitrogen flow of one bubble per second, with any sulphide product trapped by a cadmium acetate train. These last experiments were sampled frequently (some at daily intervals) for optical density and sulphate concentration measurement and precipitation of sulphate as barium sulphate for isotope measurement. Experiments ran for 10 to 42 days, depending on previous experience of reaction rate for the particular site. Temperature was controlled at 15 ºC, the median value of the water temperature of field samples. At the end of each reaction, samples were purged through a cadmium acetate train, the precipitated sulphide collected, and the remaining solution in the reaction flask acidified to pH <2 and purged again to collect any further hydrogen sulphide as cadmium sulphide.

Sulphate in solution (assumed to be 3 times the measured S concentration) was determined using ICP-AES techniques. Reproducibility of the sulphur measurement is ± 1.3 %. The $\delta\,^{34}S$ values for sulphate and sulphide sulphur were determined by isotope ratio mass spectrometry of the sulphur dioxide formed by oxidation of the sulphur compounds in the presence of vanadium pentoxide and copper turnings. Mass spectrometric determination of $\delta\,^{18}O$ in the sulphate ion was by analysis of the CO_2 product formed on oxidation of barium sulphate in the presence of graphite followed by conversion of the CO-CO_2 mixture to CO_2. Accuracy of oxygen and sulphur isotope measurements is better than ± 0.2 ‰.

For two sets of experiments carried out with continuous purging, and frequent sampling, the measured loss of sulphur, assumed to be sulphate, varies from 0 % to 20 %, depending on the sediment site and whether the medium was corrected for salt concentration. There was no change in sulphate loss, consistent between sampling sites, and which reflected that the first experiment (Exp. 3) was observed for 42 days and the second (Exp. 6) for only 22 days. At the end of the observation time each reaction was purged first at the pH of reaction ($\cong 7$) and then under acid conditions. The reaction was then considered to have reached the infinity condition. Values of $\Delta\,^{34}S$ at t_∞ range from −10 to +26‰. The original $\delta\,^{34}S_{sulphate}$ is close to zero in each experiment. From this it can be implied that in at least some experiments, sulphate ion has undergone reduction during the period of observation to an extent that seems not to be reflected in any observed loss of sulphate ion at the end of reaction. The negative values for $\Delta\,^{34}S$ derive form isotopically heavy sulphide, not from sulphate at less than its initial value. It is further observed that there are good straight line relationships between each of $\delta\,^{34}S_{sulphate}$ and $\delta^{34}S_{sulphide}$ in turn, and $\Delta\,^{34}S$ for each of 18 reactions, some of which have been sampled only at the beginning and end of the reaction period.

Graphing of sulphate concentrations (actually 3 x sulphur concentration in ppm) and $\delta\,^{34}S$ ‰ for each of 12 reactions produced a repeated picture of sulphate concentrations and isotope values cycling as a function of sampling time. Overall the sulphate concentrations move to slightly lower values and the isotope values tend to increase as observation of the reaction proceeds. The variations in sulphate concentrations throughout reaction can be up to 30 % and a 2.0 to 3.0 ‰ fluctuation in $\delta\,^{34}S_{sulphate}$ is not uncommon. These deviations from the expected linear

relationships are far outside the limits of error in experimentation and their reproducibility under varying experimental conditions over five years are a strong indication that some cycling of reactions is happening in these sedimentary microcosms. The first candidate for investigation is the possibility of bacterially promoted re-oxidation of hydrogen sulphide in the anaerobic environment of the reaction system. Optical density readings of the reaction medium, made at the time of each sampling, exhibit the cycling characteristics of the two properties already described. This is apparent during the exponential growth phase which follows a lag phase of some 6 to 12 days, depending on the origin of the sediment.

The most recent experiments have been designed to allow for the sampling of sufficient sulphide to produce, as the reaction proceeds, precipitates of cadmium sulphide large enough for isotope analysis. Sediment was used at concentrations of 50 g L^{-1}. One experiment in 2 L of medium was purged with nitrogen initially and sampled under positive nitrogen pressure but not continuously purged. The second experiment was set up in 34 individual vessels, each containing 200 mL of media solution from the same source as the 2 L sample and sealed after nitrogen purging. One bottle of reaction sample was sacrificed at each sampling time. Sampling from each bottle for sulphate and optical density measurements was achieved without ingress of air and the remainder of the sample was injected with cadmium acetate to precipitate any sulphide present. Sampling of both experiments continued daily for 28 days and thereafter at greater intervals until the reaction was stopped at 67 days. The fundamental characteristics of these reactions are similar to those observed for previous reactions.   The production of substantial quantities of hydrogen sulphide, present in the vapour head in each vessel and in solution, either as dissolved H_2S or as HS^-, has allowed the meaningful measurement of sulphur concentration in solution before and after the precipitation of sulphide.

The implications of these observations for the interpretation of the reactions taking place in the presence of sulphur reducing bacteria leads to the possibility that further exploration of the system will confirm a chemistry which is as yet speculative.

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